REMARKS

Rejection Under 35 USC 112, Second Paragraph

Claims 2-8, 13-19, 21-27, 33-36, 38-40, 42-47, 53-56, 59-64, 71-75 and 81-83 have been rejected under 35 USC 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. More specifically the Patent Office states:

> regarding Claims 2, 21, 42, and 59, the phrase "characterized" renders the claims indefinite because it is unclear whether the limitations following the phrase are part of the claimed invention.

In response to this rejection, the objectionable phrase "characterized by the ability" has been deleted from Claims 2, 21, 42, and 59, and the phrase "wherein the ubiquitin fusion protein has the ability..." replaced therefore. It is Applicants' intent that the limitations following the amended phrase are part of the claimed invention. If these amended claims are determined unclear, Applicants' request guidance on preferred phraseology to indicate Applicants' stated intent.

Regarding Claim 83, the Patent Office further states:

> it is not clear exactly how and where the ubiquitin moiety is modified, is the modification on the N-terminal of the C-terminal amino acid of ubiquitin moiety? Is there an insertion, substitution or deletion of certain amino acid residues to avoid said cleavage?

This rejection is respectfully traversed. As stated in the Application on page 14, line 24-33, such fusion proteins can be made resistant to ubiquitin-specific proteases, e.g., by altering residues at the C-terminus of ubiquitin. A variety of possible modifications are well known to one of skill in the art. The Application puts forth as example altering the identity of the amino acid at position 76 of ubiquitin (e.g., from glycine to valine or cysteine) as such a modification. The Claim is intended to include all such possible modifications to the

ubiquitin moiety which render the ubiquitin fusion protein non-cleavable. It is not necessary to specify each such possible modification in order for the claims to be considered definite. As recited in the MPEP 2173.04, breadth is not indefiniteness:

> Breadth of a claim is not to be equated with indefiniteness. In re Miller, 441 F.2d 698, 169 USPQ 597 (CCPA 1971). If the scope of the subject matter embraced by the claims is clear, and if applicants have not otherwise indicated that they intend the invention to be of a scope different from that defined in the claims, then the claims comply with 35 USC 112, second paragraph.

Applicants' respectfully submit that the scope of the subject matter embraced by the claim (and amended claims...) is clear.

Rejection Under 35 USC 102 (e)

Claims 2, 3, 8, 13, 27, 42, 43, 53, 70, 74, and 75 have been rejected under 35 USC 102(e) as being anticipated by Rechsteiner et al. (US Patent 5,763,225). More specifically the Patent Office states:

> Rechsteiner et al. teach the synthesis and recovery of ubiquitin-carboxy extension peptides wherein the peptides contain two to forty amino acid residues, (see abstract, and claims, especially claim 1). Rechsteiner et al. disclose that the ubiquitin fusion proteins of their invention may be isolated and purified without cleavage of the peptide, and may be possible to obtain anti-peptide antibodies by immunizing with the ubiquitin-carboxyl terminal directly cross linked to a suitable carrier, (see column 8, lines 52-57).

In connection with the rejection of Claims 2, 3, 8, 13, and 75, this rejection has been obviated by the amendment of Claim 2 to include the limitation that the epitope containing segment is not efficiently cleaved from the ubiquitin fusion protein by exposure to ubiquitin-specific proteases *in vivo*. Support for this amendment is found in the Application on page 14, line 16-25, which recites:

> Generally, the fusion of peptides to the C-terminus of ubiquitin generates a construct which is cleavable, *in vivo*, by ubiquitin-specific proteases. It is well-established that such ubiquitin-specific proteases cleave ubiquitin fusions after a C-terminal residue (residue 76), thereby releasing the C-terminal peptide. The present invention also encompasses ubiquitin fusion proteins which have been modified such that the fusion is not efficiently cleaved by ubiquitin-specific proteases.

It is clear from the disclosure of Rechsteiner et al. that the ubiquitin fusion protein disclosed was intended to have a C-terminal extension which is susceptible to cleavage by ubiquitin-specific proteases. Modifications to make the ubiquitin fusion protein non-cleavable are not discussed in the disclosure, except in relation to specify conditions to avoid, for example, the limitation of Claim 1 that "the N-terminal amino acid of the (C-terminal extension) peptide is any amino acid except proline". This limitation was obviously included in the Claim to preclude ubiquitin fusion proteins which were resistant to cleavage (see Rechsteiner et al. column 3, line 60 to column 4, line 4). Therefore, Applicants' amended Claim 2, and the claims dependent therefrom, and Claim 75 which refers to Claim 2, are not anticipated by the disclosure of Rechsteiner et al.

In connection with the rejection of Claims 27, 42, 43, 53, 70, and 74, as being anticipated by Rechsteiner et al., this rejection is respectfully traversed. As stated by the Patent Office, "Rechsteiner et al. teach the synthesis and recovery of ubiquitin-carboxy extension peptides". Claims 27 depends from Claim 21, which specifically recites the ubiquitin is fused to two or more non-contiguous epitope-containing segments. As Applicants' explained in the previously submitted Amendment mailed October 26, 1999, the term "non-contiguous" indicates that the epitope-containing segments are located at different sites of the ubiquitin protein. The ubiquitin fusion protein disclosed by Rechsteiner et al. contains an epitope containing segment fused at only one site of the ubiquitin protein. Therefore,

Rechsteiner et al. does not anticipate Claim 21 or claims depending therefrom.

Claims 42, and Claims 43, and 53 which depend therefrom, specifically recite:

> "A ubiquitin fusion protein fused to a single epitope-containing segment comprising two or more identical or non-identical epitopes, the epitope-containing segments being fused to ubiquitin at fusion sites selected from the group consisting of the N-terminus and an internal fusion site,..."

Rechsteiner et al. teaches a peptide fused to the C-terminus of ubiquitin. Rechsteiner et al. does not teach a ubiquitin fusion protein having fusions located at the N-terminus of ubiquitin or at an internal fusion site of ubiquitin, and thus does not anticipate Claims 42 or the claims depending therefrom. Similarly, Claim 70 depends from Claim 59, which specifically recites the limitation "the epitope-containing segment being fused to ubiquitin at the N-terminus of ubiquitin", therefore, the disclosure of Rechsteiner et al. does not anticipate Claim 59 or claims depending therefrom.

Claims 74 specifically recites limitations to the ubiquitin fusion proteins described in Claims 21, 42, or 59, discussed above in connection with rejection of Claims 21, 42, 59, and claims depending therefrom. These limitations are equally relevant to Claim 74, and thus the disclosure of Rechsteiner et al. does not anticipate Claim 74.

The present invention would not have been obvious in light of Rechsteiner et al. for reasons stated below in connection with the rejection of Claims under USC 103(a) as unpatentable over van der Zee et al. in view of Vannier et al., and over Wittliff et al. in view of van der Zee et al. Rechsteiner et al. does not teach ubiquitin as an appropriate carrier protein to generate immunogenicity to the carboxyl-terminal extended peptides. This is evident from the following statements made in the Rechsteiner et al. disclosure:

> it may be possible to obtain anti-peptide antibodies by immunizing with ubiquitin-carboxyl terminal extended peptides directly cross-linked to a suitable carrier protein. (column 8, line 52-55)

Rejection Under 35 USC 102(b)

Claims 2, 3, 8, 13, 27, 42, 43, 53, 70, 74, and 75 have been rejected under 35 USC 102(b) as being anticipated by Wittliff et al. (1990). More specifically the Patent Office states:

> Wittliff et al. teach the expression and characterization of an active human estrogen receptor as a ubiquitin fusion protein from E. coli, ...

This rejection is respectfully traversed. Claims 2-3, 8 and 13 specifically recite "A ubiquitin fusion protein comprising ubiquitin fused to a single epitope-containing segment, the epitope containing segment comprising two or more identical epitopes...". As stated in the above quoted passage, Wittliff et al. teaches ubiquitin fused to an active human estrogen receptor. Wittliff does not teach ubiquitin fused to two or more identical epitopes and therefore does not anticipated Claims 2-3, 8 or 13.

Claim 27 depends from Claim 21, which specifically recites "A ubiquitin fusion protein comprising ubiquitin fused to two or more non-contiguous epitope-containing segments." As discussed above in connection with the rejection of Claims as anticipated by Rechsteiner et al., the term "non-contiguous" indicates that the epitope-containing segments are located at different sites of the ubiquitin protein. The ubiquitin fusion protein disclosed by Wittliff et al. contains an epitope containing segment fused at only one site of the ubiquitin protein. Therefore, Wittliff et al. does not anticipate Claim 21 or claims depending therefrom.

Claim 42, and Claims 43, and 53 which depend therefrom, specifically recite the limitation that the epitope-containing segment is fused to either the N-terminus or an internal fusion site of ubiquitin. Wittliff et al. teaches the human estrogen receptor fused to the C-terminus of ubiquitin. Wittliff et al. does not teach a ubiquitin fusion protein having fusions located

at the N-terminus of ubiquitin or at an internal fusion site of ubiquitin, and thus does not anticipate Claims 42 or the claims depending therefrom.

Claim 70 depends from Claim 59, which specifically recites the limitation "the epitope-containing segment being fused to ubiquitin at the N-terminus of ubiquitin." As discussed directly above, Wittliff et al. does not teach such a ubiquitin fusion protein, and therefore does not anticipate Claim 70.

Claims 74 and 75 specifically recite the limitations to the ubiquitin fusion proteins described in Claims 2, 21, 42, or 59, discussed above in connection with rejection of Claims 2, 21, 42, 59, and claims depending therefrom. The limitations are equally relevant to Claims 74 and 75, which are thus also not anticipated by the disclosure of Wittliff et al.

Claims 2, 3, 8, 13, 27, 42, 43, 53, 70, 74, and 75 have been rejected under 35 USC 102(b) as being anticipated by Vannier et al. (1996). More specifically the Patent Office states:

> Vannier et al. teach the expression of the extracellular domain of human follicle stimulating hormone receptor (hFSHR) in E. coli as a ubiquitin fusion protein, (see abstract). Vannier et al. disclose that the immunization of mice with Ub-hFSHR allowed the preparation of high affinity anti-receptor monoclonal antibodies, this Ub-hFSHR fusion protein also provoked the formation of anti-receptor antibodies in monkeys ... The Ub-hFSHR disclosed by Vannier et al. meets all the limitations in instant claims 2-3, 8, 13, 27, 42-43, 53, 70, 74-75.

This rejection is respectfully traversed. Vannier et al. teaches a fusion protein comprised of ubiquitin fused to extracellular domain of human follicle stimulating hormone receptor (hFSHR). Although the disclosure of Vannier et al., and also the disclosure of Loosefelt et al. referenced as a source of information for the construction of this fusion protein, do not specifically disclose the construction of this fusion protein, one of skill in the art would reasonably conclude from the nomenclature used (Ub-hFSHR(23-358) that the fusion protein is

comprised of amino acids 23-358 of hFSHR fused to the C-terminus of ubiquitin. The fusion protein of Vannier et al. therefore shares the same relevant characteristics as the fusion protein disclosed by Wittliff et al., discussed above. Therefore, the disclosure of Vannier et al. does not anticipate Claims 2, 3, 8, 13, 27, 42, 43, 53, 70, 74, and 75 for the same reasons discussed above in connection with the rejection of said claims over the disclosure of Wittliff et al.

Rejection Under 35 USC 103(a)

The rejection of Claims 15, 35, 55, 72, 81, and 83 made under 35 USC 103(a) as being unpatentable over van der Zee et al. in view of Vannier et al. has been maintained. In connection with this rejection, the Patent Office has stated previously:

> it would have been obvious to one of ordinary skill in the art to modify the GnRH fusion protein taught by van der Zee et al. by fusing GnRH to ubiquitin as taught by Vannier et al. because ubiquitin is a small highly conserved protein that is found in all eukaryotic cells and that does not induce immune response in animals.

In connection with the rejection of Claim 81, Claim 81 has been amended to specifically recite that the ubiquitin fusion protein is non-cleavable by a ubiquitin-specific protease. This amendment obviates the rejection for the reasons stated in the following paragraph regarding the traversal of this rejection as it applies to Claims 15, 35, 55, 72, and 83.

In connection with the rejection of the remaining claims, this rejection is respectfully traversed. The ubiquitin fusion protein of the present invention provides the double advantage of a) conferring antigenicity to a relatively small peptide sequence, which allows specific targeting of the immunogenic response to one or two epitopes of a protein, and b) generating minimal if any immune response to the ubiquitin carrier, which eliminates the need to differentiate between and or separate the immunogenic response to the fusion and the response to the peptide sequence. Relatively small peptides (e.g., the epitopes

of Applicants' invention) are non-immunogenic in the absence of a carrier. This is in contrast to large peptides/proteins which are often significantly immunogenic in the absence of carrier. To confer antigenicity to a short, otherwise non-antigenic peptide sequence, standard practice in the art would be to attach the peptide sequence to a highly antigenic molecule. The ability of ubiquitin to function well as a carrier for the epitopes specified in Applicants' invention could not have been predicted by one of skill in the art with any degree of certainty, because ubiquitin is such a highly conserved protein and thus is minimally antigenic itself. In addition, the ability of ubiquitin to remain non-immunogenic in the context of the fusion protein which is antigenic to the fused epitopes, could not have been predicted by one of skill in the art with any degree of certainty.

As discussed above, Vannier et al. teach a ubiquitin-hFSHR fusion protein, the very large hFSHR fragment (amino acids 23-358) is fused to the C-terminus of ubiquitin, and is susceptible to cleavage by ubiquitin-specific proteases, to generate monoclonal antibodies in mice to FSHR. The ubiquitin portion of the fusion protein taught by Vannier et al. does not confer increased antigenicity to the fused hFSHR protein due to rapid cleavage of the C-terminal fusion upon injection by ubiquitin-specific proteases. The C-terminal fusion is relatively large in size, and is intrinsically immunogenic, whether in the form of a fusion protein or as an isolated polypeptide.

In contrast to the disclosure of Vannier et al., van der Zee et al. teaches the fusion of the short GnRH decapeptide to the highly immunogenic carrier P-fimbriae. The short GnRH decapeptide is specifically described as non-immunogenic in mammals (see first paragraph of van der Zee et al.). Neither disclosure, Vannier et al. or van der Zee et al., provides information which would suggest to one of skill in the art that attachment of a non-immunogenic short peptide such as GnRH to a

non-immunogenic carrier ubiquitin would produce a fusion protein which is immunogenic for the short peptide.

Claims 4-7, 14-19, 21-26, 33-36, 38-40, 44-47, 54-56, 71-73 and 81-83 have been rejected under 35 USC 103(a) as unpatentable over Wittliff et al. in view of van der Zee et al. More specifically the Patent Office states:

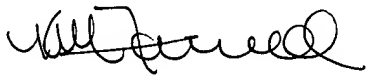
> it would have been obvious to modify the GnRH fusion protein taught by van der Zee, by generating GnRH as a ubiquitin fusion protein, using the teachings of Wittliff et al. because Wittliff et al. teach that the expression of proteins as ubiquitin fusions facilitates stabilization and increases efficiency of translation, and the attachment of ubiquitin promotes proper folding, thus preserving the protein's biological activity ...

This rejection is respectfully traversed. Wittliff et al. disclose a ubiquitin fusion protein fused to the complete amino acid sequence of the human estrogen receptor (described as a 40-45 kD protein, see page 22019, third paragraph, second column) at the C-terminus of ubiquitin. This fusion protein is similar to the fusion protein of Vannier et al. in that the fused protein is expected to be antigenic in the absence of carrier. One of skill in the art would not have predicted with any degree of certainty that the ubiquitin fusion protein of the present invention would be antigenic for the fused epitope while remaining non-immunogenic for the ubiquitin sequences, from reading the disclosures of Wittliff et al. and van der Zee et al. for the same reasons discussed above in connection with the rejection of claims over Vannier et al. in light of van der Zee et al.

Summary

In light of the above amendment and remarks, reconsideration of the subject patent application is respectfully requested.

Respectfully submitted,



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